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Agricultural Research

FORUM

Improving Animal Disease Diagnosis

Cattle, swine, and sheep provided over $50 billion in revenue to the livestock industry last year.

But millions of dollars are siphoned off these earnings every year by debilitating or fatal animal diseases. In addition to losses caused by death, diseases prevent animals from producing maximum amounts of meat, milk, and fiber. The market for livestock is also reduced because of export restrictions.

ARS animal researchers study infectious diseases to help ranchers produce healthier livestock. The first step toward controlling or eliminating a disease is to diagnose it.

Sir Macfarlane Burnet, a 1960 Nobel laureate for his work on the immune system, pointed out two key questions that we must answer to understand and eventually control any infectious disease:

What are the means by which an infectious agent persists in nature from one generation to the next?

What are the susceptible host species and the means by which transmission occurs?

Recent advances in molecular biology promise more revealing answers to these questions than ever before. New diagnostic tools such as DNA probes, competitive inhibition enzyme-linked immunosorbent assays, and monoclonal antibodies are fast replacing standard complement fixation and indirect immunofluorescence tests in fundamental research, diagnostic veterinary medicine, and epidemiology.

Such tools have ushered out the generalist in animal disease research. Fifty years ago, a scientist could work in a variety of fields like nutrition, infectious diseases, and toxicology. Now it takes more funds and more sophisticated procedures to solve problems. This forces researchers to specialize. Government and university scientists frequently combine both their intellectual and capital resources to master the technology that allows them to answer in-depth questions about disease transmission.

The keys to these tools—and the additional advances that are underway—are their sensitivity and specificity. Sensitivity refers to the ability of a test to identify those individuals that are infected, while specificity is the ability to identify those that are *not* infected.

With a DNA probe, for example, researchers can identify animals that are carrying—and potentially spreading—a disease without showing clinical signs of illness. Identifying carriers is paramount in disease control, because these animals are a major reservoir of infectious diseases.

Examples of persistent infections discussed in this issue include malignant catarrhal fever (caused by a herpesvirus), anaplasmosis (caused by a microscopic organism called a rickettsia), babesiosis (caused by a protozoan), and bovine spongiform encephalopathy and scrapie (causes not yet known).

Scrapie infection in sheep is an example of how the lack of a diagnostic test for identification of carrier animals can handicap eradication. Millions of dollars have been spent on scrapie control without any real success.

But even as we appreciate the potential of the new biological tools, we realize that they, too, will become obsolete. Existing tools have led to diagnostic tests for malignant catarrhal fever and anaplasmosis but have not yet revealed the secrets of spongiform encephalopathies.

More sensitive tools, such as polymerase chain reaction and restriction fragment length polymorphism, are at the leading edge of tomorrow's diagnostic research. While it is hazardous to speculate, early in the next century imaginative molecular biologists may develop something as simple as a dipstick test to diagnose most infectious diseases in a matter of minutes.

In diseases transmitted by ticks, biting flies, and other vectors, molecular probes will identify the species and geographic strains of the disease agents. Sensitive probe methodologies will also be at the forefront in developing and testing vaccines.

But the key to effective disease control will always be the curious scientist—only the tools will change.

John Gorham
ARS Veterinary Medical Officer
Pullman, Washington

Agricultural Research

At Pullman, Washington, and Moscow, Idaho, ARS scientists and cooperating researchers are developing new diagnostic tests for livestock diseases.
Photo by Scott Bauer. (K5470-17)

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Editor: Lloyd McLaughlin (301) 344-2514
Associate Editor: Linda McElreath (301) 344-2536
Art Director: William Johnson (301) 344-2561
Contributing Editor: Jeanne Wiggen (301) 344-2502
Photo Editor: John Kucharski (301) 344-2900
Assoc. Photo Editor: Anita Daniels (301) 344-2956

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Mike Espy, Secretary
U.S. Department of Agriculture

R.D. Plowman, Acting Assistant Secretary
Science and Education

Essex E. Finney, Acting Administrator
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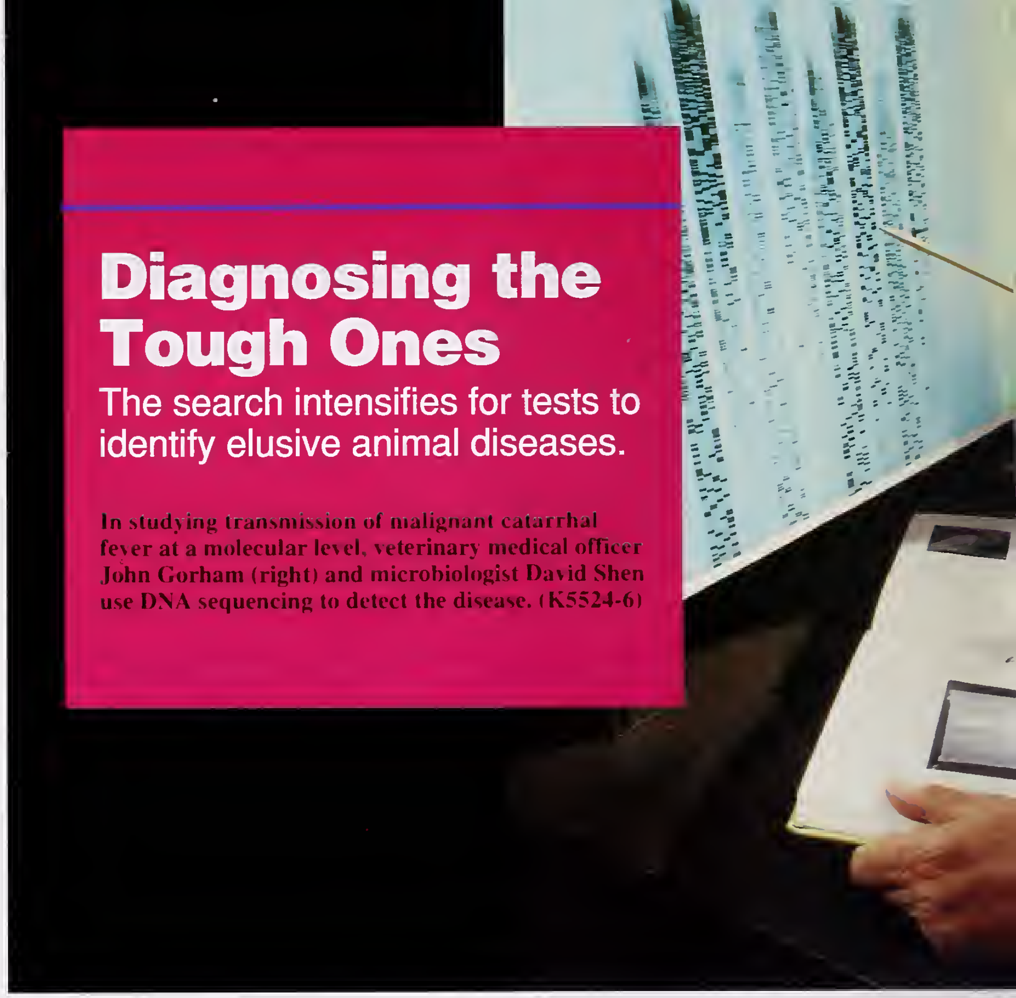
Information Staff



Diagnosing the Tough Ones

The search intensifies for tests to identify elusive animal diseases.

In studying transmission of malignant catarrhal fever at a molecular level, veterinary medical officer John Gorham (right) and microbiologist David Shen use DNA sequencing to detect the disease. (K5524-6)

Accurate diagnosis of animal disease can be a pocketbook issue to producers.

In 1932, a swine disease appeared in California that was thought—incorrectly—to be foot-and-mouth disease. It was not correctly diagnosed until the next year. By then, 20,000 pigs and their barns had been destroyed to stem the outbreak.

"Correct diagnosis is the key to disease control," says veterinary medical officer John Gorham, who is with USDA's Agricultural Research Service. Gorham heads up the ARS Animal Disease Research Unit, which is located at Washington State University (WSU) in Pullman and at the University of Idaho in Moscow.

To improve disease detection, scientists there are developing new, state-of-the-art tests. The main focus is on diseases that persist in an animal's tissues even after the animal seems to recover. Such animals become lifelong carriers that can spread the disease to others.

Accurate, reliable tests have yet to be developed for many diseases. Without them, ranchers and disease control agencies spend millions of dollars each year to treat sick animals, quarantine potential carriers, and destroy disease-spreading organisms such as ticks and flies. Ranchers lose money not only from the actual death of livestock, but also because they can't export animals that might carry disease to other countries.

"The goal is to get simple tests that can be conducted in the field on large numbers of animals. Once we can reliably detect a disease, we can work towards a vaccine to prevent it," Gorham says.

Using the latest tools from molecular biology, ARS scientists have recently come up with rapid tests for two serious cattle diseases.

SCOTT BAUER

Using electrophoresis, Hong Li analyzes proteins in malignant catarrhal fever virus in order to develop a more specific diagnostic test. This virus causes an often fatal disease in cattle. (K5519-2)

Malignant Catarrhal Fever

While the present extent of malignant catarrhal fever is unknown because of the lack of practical detection procedures, two things are quite certain: It is one of several cattle diseases caused by a herpesvirus, and it is often fatal.

And now, for the first time, malignant catarrhal fever can be diagnosed.

ARS microbiologist David Shen, doctoral student Hong Li, and WSU virologist Timothy Crawford have developed the first reliable diagnostic test. It relies on two techniques: competitive inhibition enzyme-linked immunosorbent assay (CI-ELISA) and monoclonal antibodies.

In a typical ELISA, the malignant catarrhal fever virus is applied to the bottom of a plastic plate. Blood serum from a cow to be tested is then placed on the plate. If the cow has the disease, its serum contains antibodies that bind to proteins found in the

SCOTT BAUER

virus. If that happens, the color changes on the plate.

A problem is that the cow's blood contains many antibodies—not just those generated in response to the malignant catarrhal fever virus. So even if the test is positive, it is impossible to be sure that the antibodies are specifically recognizing the virus.

That's where monoclonal antibodies come in. These antibodies, which are made in the laboratory, are more specific than the antibodies normally made by a cow. Monoclonal antibodies respond to only one small part of a specific protein.

If the cow's blood doesn't have natural antibodies to malignant catarrhal fever virus, the monoclonal antibody will form a bond to the ELISA plate that is indicated by a color change.

But if the cow's blood does contain antibodies to the virus, the monoclonal antibody would not have a chance to bind. The CI-ELISA would then show no color change, indicating that the cow does indeed have the virus.

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In a red-lit darkroom, assistant Kathleen Hendrix and entomologist Michael Coan observe ticks' behavior on a feeding device that mimics host animal's skin. (K5520-10)

Shen and Li produced over 200 monoclonal antibodies against different proteins of the virus before they found one that could be used to diagnose it. They have developed the test and may apply for a patent.

Two forms of malignant catarrhal fever exist, one in domestic sheep and one in African wildebeests. Both species of animals carry—but do not show signs of—the disease.

Cattle seem to contract the disease from sheep in the United States and from wildebeests in Africa. And sheep may give the disease to other wild ruminants. Still, it's a mystery how the virus spreads from one animal to another.

Using another biotechnology technique called polymerase chain reaction (PCR), the team developed a test to study transmission of malignant catarrhal fever at the molecular level. Polymerase chain reactions make copies of the genetic material so that extremely low levels of DNA can be amplified and detected.

"PCR makes it possible to detect the virus' DNA sequence in newborn lambs and ewe's milk. This has given us some preliminary evidence that lambs can be infected with the virus from their mothers," Shen says.

"We are studying sheep to find out how they transmit the disease to cattle. We are also interested in why sheep don't get sick from the virus."

Anaplasmosis—Practical Test on the Way

The actual number of cattle infected with anaplasmosis—a disease caused by a tickborne parasite that invades red blood cells—isn't known, because an accurate diagnostic test for use in the field isn't available. However, anaplasmo-

sis is thought to cost ranchers up to $300 million annually.

In 1988, Will Goff helped develop a DNA probe to identify the parasite in ticks, biting flies, and the host cattle. [See "Test for Tickborne Cattle Disease," *Agricultural Research*, September 1988, p. 4.] This probe still forms one of the cornerstones of *Anaplasma* research.

These probes allow scientists to search out the genetic material, or DNA, of the parasite in ticks or cattle.

But, "while DNA probes for infectious diseases are very good from a research standpoint, they are not feasible for routine testing of large numbers of animals," says ARS veterinary pathologist Don Knowles.

So Knowles and researchers at Washington State University looked for a more practical test. A WSU molecular biologist, Elizabeth Visser, found the key—a protein they named "major surface protein 5," or MSP-5.

"The importance of this protein is that it occurs in all known *Anaplasma* species," Knowles says. "We don't have to work on each one separately."

A monoclonal antibody that binds to MSP-5 was developed by WSU collaborators.

Knowles and WSU researchers developed the monoclonal antibody-based test and have applied for a patent. VMRD, a company in Pullman, has licensed the test. A commercial kit could be available as soon as July 1995.

Searching for a Babesiosis Vaccine

Starting with the earlier advances in anaplasmosis, Goff has been searching since 1982 for an improved vaccine for bovine babesiosis, another tickborne disease caused by a protozoan that attacks red blood cells.

Bovine babesiosis was eliminated from the United States in the 1940's by eradicating the *Boophilus* ticks that spread it. But only constant vigilance at the U.S.-Mexico border is keeping the ticks at bay [See "Cattle Take a Dip at the U.S. Border," *Agricultural Research*, April 1994, pp. 7-9.].

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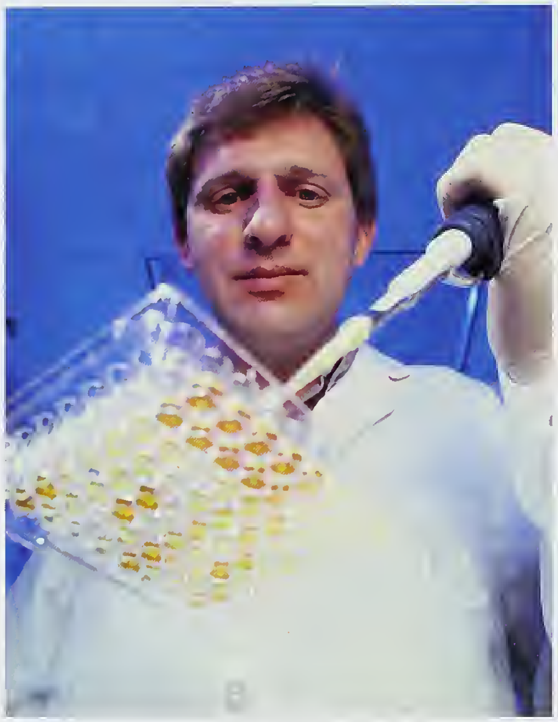

Technician Lowell Kappmeyer uses a monoclonal antibody-based test to detect *Anaplasma* species in cattle serum samples. (K5521-12)

There are currently no reliable, safe vaccines available for use in the United States. Cattle can't be exported to countries that have babesiosis—which includes almost all tropical and subtropical countries—because the cattle would not survive once they got there.

Typically, a vaccine induces the production of antibodies in an animal against a disease. Antibodies recognize proteins found in or on parasites, bacteria, and viruses, and they destroy the microorganisms.

But Goff observed that the *Babesia* parasite seems to have outsmarted this part of an animal's defenses.

"Cattle produce antibodies to several *Babesia* proteins, but they don't appear to offer protection from the parasite. This may be an evolutionary strategy to protect the parasite from the cattle's immune system," Goff says. The same phenomenon has been discovered in malaria, another blood parasitic disease. Malaria is transmitted to humans by mosquitoes.

So researchers are looking for other ways that cattle fight against these parasites.

The most important discovery so far is that a molecule called gamma-interferon seems to activate specialized blood cells called macrophages. Activated macrophages destroy the parasites, either by engulfing and absorbing them, or by producing molecules that are toxic to *Babesia*.

Because spleen cells produce more gamma-interferon than other cells in the body, Goff hopes the key to a vaccine is in this organ. His newest project will look at the role of the spleen in fighting *Babesia*.

Fooling Ticks into Feeding

"Historically," says ARS entomologist David Stiller, "research has looked at the relationships between parasites and their livestock hosts, or between ticks and livestock. However, it is rapidly becoming possible to investigate the complex interactions among all three parts of the system—the parasites, the ticks that carry them, and the hosts."

Studies of ticks and how they carry and transmit blood parasites have long been hampered by the lack of molecular diagnostic tools.

Practical problems were also common, because it has been difficult to raise *Anaplasma* parasites in the laboratory.

And ticks, it turns out, are finicky feeders; they must generally obtain their blood meals by feeding on live animals.

But biotechnology tools such as DNA probes and PCR—along with an innovative new feeding system for ticks—are allowing scientists to overcome many of these problems for the first time.

The key to the feeding system is a membrane derived from cow intestines. It was recently developed by Samuel Waladde in Kenya and modified by Stiller and ARS entomologist Michael Coan.

To mimic the skin surface of the host, the membrane is covered with cattle hair, cattle skin secretions, and tick feces. It is then heated to body temperature. Ticks sitting on top of the membrane put their mouth parts through the membrane to feed on cattle blood that is stored underneath.

The feeding system, along with the biotechnology tools, should allow scientists to answer two important questions: How few parasites are required to establish infection in ticks, and how many parasites are transmitted to cattle when infected ticks feed?

Stiller can now put precise concentrations of parasites in the feeding chamber's blood supply. Then he can measure how many parasites the ticks take up when they feed. From this he can determine the minimum levels of parasites in a cow's blood that it takes to infect different species of ticks.

By then feeding the ticks on "clean" blood, he can determine how many parasites the ticks spit back into a cow. "Although this system won't eliminate the use of all animals in research, it will permit us to measure the number of parasites that ticks introduce into vaccinated cattle. This information can be used to test and standardize vaccines," Stiller says.

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Rocky Mountain wood ticks feed on an "artificial cowhide" made from intestinal tissue covered with hair, feces, and skin secretions. The membrane normally fits over a small, cylindrical chamber filled with cattle blood, but it was removed and flash-frozen for observation. (K5522-3)

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ARS entomologists David Stiller (right) and Michael Coan examine a feeding chamber designed for hard-bodied ticks. A moat around the table edge prevents live ticks from crawling away. (K5518-13)

"The system mimics the natural relationship between the tick and the host better than anything we've used previously," he adds. "We're just getting started, and it's very exciting."

Bovine Spongiform Encephalopathy

Diagnostic tests and vaccines provide tangible, rewarding results after years of research. But even basic facts about some animal diseases, such as bovine spongiform encephalopathy (BSE), is still unknown.

BSE is a degenerative disease of the central nervous system that has killed over 125,000 cattle in Britain since 1985.

Although BSE has not been detected in the United States, ARS scientists are investigating the disease to assist the USDA's Animal and Plant Health Inspection Service and British authorities with prevention and control efforts.

The outbreak in Britain is believed to have arisen from a now-discontinued feeding practice. Rendered meat scraps used as protein supplements are believed to have contained meat from sheep that were infected with a related disease called scrapie.

Because the cattle that ate this feed developed BSE, and because the number of new cases has declined since the use of rendered protein supplements in cattle feed was discontinued, scrapie has been implicated in the transmission of BSE. But the causes of both diseases are unknown, and the diseases can't be detected easily.

ARS microbiologists Mark Robinson and Katherine O'Rourke are looking for a diagnostic technique to detect BSE in live animals. Currently the only way to detect BSE or scrapie is to dissect the brain of the animal after it dies. The infected brain has "spongy" areas that give spongiform encephalopathies their name.

Robinson is exploring related diseases that occur in the United States: scrapie in sheep and transmissible mink encephalopathy, or TME, in mink.

For the first time, Robinson has experimentally infected mink with TME by feeding them cattle brains that contain BSE. "This indicates that BSE and TME are related, but we're not sure how," Robinson says.

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Entomologist David Stiller (standing) and technician Ralph Horn check research animals at the USDA holding facility housed at the University of Idaho in Moscow. An artificial tick feeding system now reduces the need for animals in tick and blood parasite studies. (K5523-7)

He has not been able to cause disease in mink by feeding them sheep infected with scrapie, which is also presumably related to BSE. "Some of the encephalopathies seem to cross the species barriers and some do not," he says.

Concern about transmission of related diseases to humans has periodically caused British consumers to avoid beef, but there is no scientific evidence to support that such transmission is possible.

"For example," says Gorham, "Australia does not have BSE or scrapie. But they have the same number of cases per capita of a human spongiform encephalopathy called Creutzfeldt-Jakob disease as do the United States and England."

Nevertheless, the importance of cattle disease research extends beyond the health of individual animals and the potential millions of dollars in savings for cattle ranchers.

Most animals, including humans, are vulnerable to some form of herpesvirus. Humans suffer from babesiosis and malaria, diseases caused by blood parasites, which are spread by ticks and mosquitoes. And researchers hope that by discovering the nature of BSE, they will provide insight into spongiform encephalopathies that affect several mammalian species, including humans.—By **Kathryn Barry Stelljes**, ARS.

Scientists mentioned in this story are with the USDA-ARS Animal Disease Research Unit. David Shen, Don Knowles, Will Goff, Katherine O'Rourke, Mark Robinson, and John Gorham are at 337 Bustad, Washington State University, Pullman, WA 99164-7030; phone (509) 335-6001, fax (509) 335-8328.

David Stiller and Michael Coan are at the Holm Research Center, University of Idaho, Moscow, ID 83843; phone (208) 885-7081, fax (208) 885-8937. ◆

Wingbeat Counter Fingers Invading Bees

Africanized honey bees in Central America beat their wings faster than domestic European bees in Arizona. That difference, thinks an Agricultural Research Service entomologist, may be a way to tell the two apart.

The current test to verify that bees are Africanized requires capturing specimens and shipping them to state and federal laboratories. There, experts measure minute differences in body part sizes.

Identification by wingbeat frequency could prove faster and cheaper and—best of all—wouldn't require handling bees that might suddenly mount an attack.

So Hayward G. Spangler tape-recorded the wingbeat frequency of Africanized honey bees that he encountered during two trips to Costa Rica. He compared these recordings to others made by European bees at the Carl Hayden Bee Research Laboratory in Tucson, Arizona.

"Costa Rican bees that were foraging for nectar or flying toward and away from their hives had a higher wingbeat frequency than Arizonan bees.

"But at times, this difference wasn't consistent. European bees at the Arizona lab beat their wings faster while robbing honey from a honeycomb," says Spangler. "And we couldn't differentiate the two bees by their wingbeats when they were seeking water or a new nesting site or when they were defending their hives.

"Both types become more agitated and beat their wings faster during a stinging attack," he says.

Spangler's next step will be to measure wingbeat frequencies of Africanized honey bees that have moved into the United States from Mexico. He says it is possible that Africanized bees have modified their behavior during their 2,200-mile migration, and their wingbeat may not be the same as those he measured in Costa Rica.

When testing stinging bees or bees flying toward and away from their hives, Spangler used an optical transducer to detect the signals of changing light intensity reflecting off the wings of a single bee. His detector was about 15 feet from the colony and aimed at a spot about 2 feet in front of the hive's entrance. The device converted the reflected light into electrical signals that were recorded on magnetic tape for laboratory analysis.

Spangler thinks these findings could aid engineers in developing an affordable device to help track the spread of Africanized bees. The detector would help identify potentially dangerous honey bee colonies near schools and playgrounds so they could be removed.—By **Dennis Senft,** ARS.

Hayward G. Spangler is in the USDA-ARS Honey Bee Research Unit, 2000 E. Allen Road, Tucson, AZ 85719; phone (602) 670-6381, fax (602) 670-6493. ◆

Biotech Probes Identify Food Yeasts

Tomorrow's bakers, brewers, and winemakers might more easily identify both helpful and unwanted yeasts in their mixing bowls or fermentation vats, thanks to work by ARS scientists.

A new yeast ID system produced by the researchers relies on matching samples of genetic material, or DNA. The DNA of known yeasts is compared to that of the unknown sample taken from within a bakery, brewery, or other foodmaking plant.

The analysis technique was developed and tested at the Western Regional Research Center, Albany, California, by A. Douglas King, Jr., and David R. Rockhold and former ARS researchers Tamás Török and Christina Royer.

"Typical schemes for identifying yeast require about 50 different tests," says King. The DNA test, he says, is simpler and easier to use.

"Unwanted yeasts in food processing don't pose a danger to health, but they can alter the flavor or character of some foods or beverages and can lower yield."

Right now, though, the technique is best suited for research labs. That's because it requires sophisticated and cumbersome scientific equipment. And, it takes too long to be practical on a busy production line—about 6 days for results. Nevertheless, within about 5 years, the test could likely be downsized and streamlined to make it a practical tool for everyday quality control.

With more work, according to King, the test could be packaged into an easy-to-use kit for making sure that a company's prized yeasts aren't working alongside unwelcome ones. That's important, because foodmakers may spend years nurturing and cultivating yeasts to get exactly the product they want.

DNA matching isn't new. But the Albany team was apparently the first to use DNA from entire yeast chromosomes to distinguish one food yeast from another. Using these chromosomes, which house yeast genes, makes the technique more accurate than relying on DNA from bits and pieces of chromosomes, says King.

The scientists have tested their procedure on 5 common food yeasts and believe that it will work just as well with the 40 or 50 other yeasts important in food processing.—By **Marcia Wood,** ARS.

For more information on patent application number 07/695,167, contact A. Douglas King, Jr., USDA-ARS Western Regional Research Center, Process Biotechnology Research Unit, 800 Buchanan St., Albany, CA 94710. Phone (510) 559-5851, fax number (510) 559-5777. ◆

Bringing Idled Land Back Into Production

Millions of acres of highly erodible land that farmers agreed to cover with grass or trees and remove from production will soon reach the ends of their 10-year contracts. Annual federal payments under the Conservation Reserve Program (CRP) will cease then.

Land owners with taxes and mortgages to pay and families to care for are looking for production systems that will meet their obligations.

And environmentalists and the taxpayers who have paid the bill for CRP would like to ensure that—to the extent possible—the benefits derived from having highly erodible lands in grass are retained. These include improved water and air quality and wildlife habitat, as well as decreased runoff, erosion, flooding, and sedimentation.

"That's a tall order, but one that our scientists are endeavoring to fill," says W. Doral Kemper, who is the Agricultural Research Service's national program leader for soil management research, in Beltsville, Maryland. "They are working with a host of cooperators that includes the USDA's Soil Conservation Service (SCS), Agricultural Stabilization and Conservation Service (ASCS), university people, and farmers.

"In addition to dozens of related studies conducted in the past," says Kemper, "more studies are now under way at many locations across the country to supply specific information that farmers need to protect their income and the environment. We need to find ways to retain the benefits—such as improved infiltration and organic matter—that accrued while the land was in the CRP."

Begun in 1985, the CRP removed 36.5 million acres from production. That represents about 8 percent of all U.S. cropland—an area as large as Iowa. Farmers were compensated with payments that averaged about $50 per acre per year for planting soil-saving grasses or trees instead of farm crops.

BRIAN PRECHTEL

Farmer and rancher John Stulp of Lamar, Colorado, inspects an erosion-control terrace planted to buffalo grass during the Conservation Reserve Program. (K5541-1)

Wind erosion has been lessened by as much as 350 million tons, reducing airborne dust blowing across several regions of the country.

Total cost of the program is estimated to be $19.2 billion over the life of all the 10- to 15-year contracts. But the value of benefits is difficult—or in some cases impossible—to measure.

Nationwide, the program has reduced soil erosion by an estimated 22 percent, or some 700 million tons every year, cutting annual sediment flow into waterways by about 100 million tons.

And wind erosion has been lessened by as much as 350 million tons, reducing airborne dust blowing across several regions of the country.

The grasses planted on land in the reserve program have provided a dramatic improvement in wildlife habitat for many species, including birds, mammals, and reptiles.

But what will happen when CRP contracts expire? A recent survey of farmers by the Soil and Water Conservation Society found that more than 60 percent of them intended to bring the reserve acreage back into crop production when their contracts expire. Most of them will plant cereal and row crops, both of which can lead to more soil erosion than cover crops if farmers use intensive tillage.

"In an ideal world, it would be possible to keep all highly erodible lands seeded to grasses and thus protect them from wind and water erosion. But, after the CRP payments end, economic necessity will force many farmers to replant with crops they can sell," says ARS soil scientist Gerald E. Schuman at the High Plains Grasslands Research Station near Cheyenne, Wyoming.—By **Dennis Senft**, ARS.

For more information, contact W. Doral Kemper, USDA-ARS National Program Leader for Soil Management, Room 232, Bldg. 005, 10300 Baltimore Ave., Beltsville, MD 20705; phone (301) 504-6065, fax (301) 504-6191. ◆

An End to Dirty Days

BRIAN PRECHTEL

John Stulp displays the root system of buffalo grass. (K5542-1

"The major benefit we have seen from the Conservation Reserve Program is that the vegetative cover has significantly improved our air quality," says John Stulp.

"South of town, land gets lighter and is more subject to blowing. Since some of the more erodible soils have been in the reserve, we've noticed southerly winds don't bring us so many dirty days."

Stulp and his wife Jane feed cattle and farm near Lamar, a small town in the southeast corner of Colorado. He says their operation's main environmental concern is wind erosion.

The Stulps have about one quarter of their acres in the CRP under four contracts. The first one will expire in the fall of 1995 and the others over the next 3 years.

"We initially signed up for the program primarily because of some environmental concerns, but also because of the economics. The steady income from the CRP over the 10-year period has helped balance the uncertain income from growing wheat on dryland," says Stulp.

"Before our first contract expires next year, we'll have to consider several factors that will determine whether we'll put the land back into production and how we'll proceed," says Stulp.

He estimates that 90 percent of CRP land in his area of the region will come back into production when contracts expire. Ten percent would stay in grassland and would be near existing native grassland, where it could be easily grazed. If contracts were extended for additional time under the same payment rate, he believes 90 percent would remain in the program.—By **Dennis Senft**, ARS.

Conservation Reserve Program

Science Looks at Idled Land, Region by Region

Northern Plains Soil and Water Research Center, Sidney, Montana.

❶ Tall wheatgrass barriers control wind erosion, trap drifting snow, and warm soil for earlier planting. Such barriers actually reshape land permanently, forming shallow terraces that reduce water erosion.

To establish tall wheatgrass barriers on CRP land, efficient ways to kill narrow strips of the existing short grass cover must be found. Herbicides hold the most promise, since, unlike tillage operations, herbicides leave crop residue in place to guard against erosion until the wheatgrass becomes established.

Scientists are also comparing spring wheat yields to potential hay yields from crested wheatgrass.

Northern Great Plains Research Laboratory, Mandan, North Dakota.

❷ Scientists will evaluate conversion alternatives being considered by farmers in this region. According to CRP contract provisions, farmers can begin working enrolled lands as early as July 1 of the year the contract expires. This summer, researchers began demonstrations aimed at showing how to maintain good soil properties after farming resumes. Past studies indicate that minimum-till or no-till will be the most effective way to produce winter wheat in this area. Scientists here continue working to find the best way to kill the grasses, keep soil in place, and maintain profitable crop yields.

Central Great Plains Research Station, Akron, Colorado.

❸ Scientists here have addressed costs and benefits of conversion from CRP to production.

Initial herbicide applications failed to completely kill the drought-stressed grasses, and repeated herbicide applications were required.

Over a 3-year period, no-till cost more to kill the grass than either reduced-till or conventional till systems that used a sweep plow to kill the grass. The reduced-till system used one or two sweeps plus contact herbicide. Winter wheat yields in no-till fields were 20 percent less than in the other two systems, due mainly to grass that continued to grow and competed with the wheat for limited water.

Some tillage operation—or a combination of tillage and herbicide—appeared to be needed to uproot and kill grasses under these conditions. Herbicides, even if applied at high rates, only control perennial grasses that were actively growing during the short fallow period. Long-term rotation studies at Akron show that perennial grasses tend to invade wheat in no-till systems.

However, in the Central Plains, rotations of wheat with other crops such as corn, sorghum, or proso millet provide opportunities for lower cost herbicidal control of perennial grasses, which makes no-till economically feasible and achieves the desired erosion control.

High Plains Grasslands Research Station, Cheyenne, Wyoming.

❹ A three-location field project examining the profit potential of native grasses has been running since 1987. The areas all receive about 14 inches of annual precipitation but vary in soil type and seasonal temperatures. The study looks at the yield potential of four native grasses grown by three cooperating farmer-ranchers in different parts of Wyoming. The lands had previously grown wheat for more than 50 years.

One ton of forage per acre per year could produce as much income as the average wheat yield of 28 bushels per acre every other year. (In Wyoming, wheat fields are kept fallow in alternating years to accumulate soil moisture.) Their plots usually produced more than 1 ton per acre—and occasionally as many as 2.

The next step is to learn how much hay from this source can enter the market without significantly depressing hay prices.

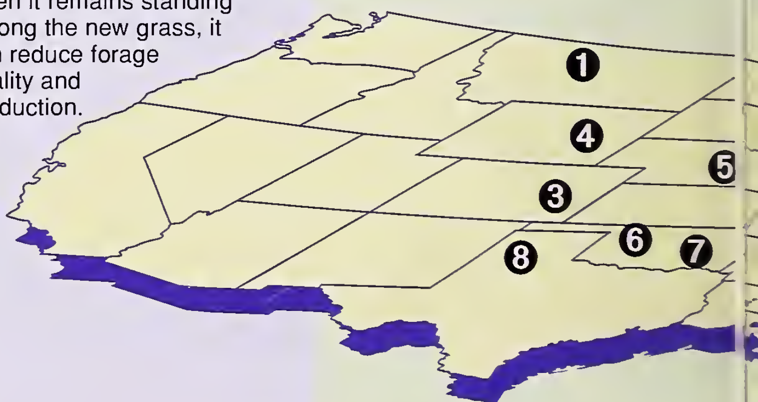
Cheyenne scientists are also looking for practices that can be used during the last year or two of the CRP contract that will enhance production and quality of forage following the CRP. Dead and decaying plant material lying on the soil surface improves the soil and water use efficiency. But when it remains standing among the new grass, it can reduce forage quality and production.

Soil and Water Conservation Research Unit, Lincoln, Nebraska.

❺ A computer program called WEPP—Water Erosion Prediction Project—may help farmers know in advance which cropping methods will best slow or control erosion. Scientists and engineers here plan to monitor selected physical, chemical, and biological soil quality indicators on actual CRP land. With this information, they can fine-tune the WEPP computer program and make it more accurately explain how fields will change under various crops and farming systems.

The sampling sites selected are representative of most soils and climatic conditions. They were part of a larger network of sites established in the late 1980's to gather information that led to WEPP.

Researchers will use a trailer-mounted rainfall simulator with ten 25-foot rotating booms to create rainstorms that will cause small-scale erosion—just like the erosion that farmers might have to cope with on a larger scale.



Southern Great Plains Range Research Station, Woodward, Oklahoma.

6 A soil scientist has begun studies aimed at finding best farming methods unique to this portion of the Great Plains. Some 70 percent of the CRP land in Oklahoma and a considerable acreage in Texas were seeded to Old World bluestem grass. Scientists will examine the possibility of keeping the grass, fertilizing it, and using it as pastureland for grazing livestock. Other options include killing the grass with herbicides and then cropping to annual winter wheat under no-till or reduced-till systems.

Grazingland Research Laboratory, El Reno, Oklahoma.

7 In a large multiagency project, scientists will evaluate reduced tillage practices in winter wheat and cotton production on land that has been on the CRP. They will also evaluate forage production in post-CRP land with both unimproved and improved grass cover. To quantify benefits that have accrued under CRP and monitor their persistence under alternate management options, they will measure soil organic matter content, infiltration, and biological indices. They will use selected management models to assess relative merits of the above cultural options and will determine the costs and benefits of the various management systems.

Conservation and Production Research Laboratory, Bushland, Texas.

8 In 1993, grain sorghum was planted on pastures that had not been cropped for at least 50 years and bore a mixture of grasses, mainly blue grama and buffalo. Scientists used three typical farming practices to establish the sorghum crop: no-till (herbicides used to kill grasses), sweep plow (to cut grass roots), and moldboard plow (to turn sod over) followed by disking. With no irrigation, crop yields ranged from a high of about 3,000 pounds per acre for the no-till to 1,100 pounds for the sweep operation and 670 pounds for the plowed and disked. The higher yields attained under no-till were a result of better water use efficiency.

So no-till appears to be the most desirable practice for farmers converting land from grass to crops. Clean tillage like plowing can wipe out, in just 2 to 3 years, all the benefits attained during the decade that CRP land was in grass—especially, improved soil properties. However, it costs about $35 an acre to kill the grass with herbicides.

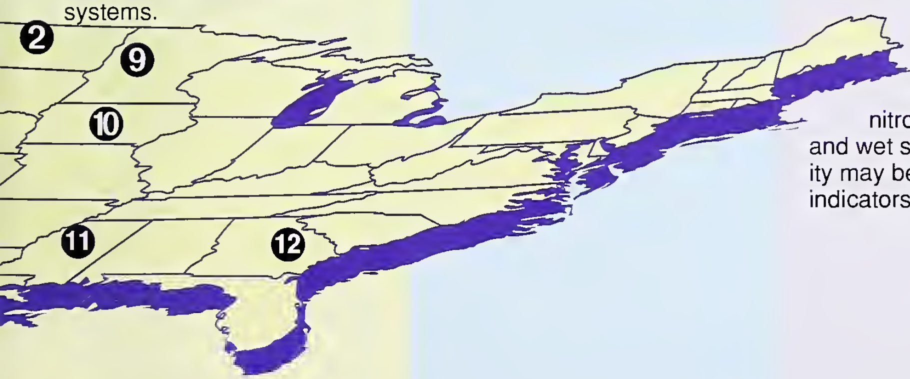
North Central Soil Conservation Research Laboratory, Morris, Minnesota.

9 A Morris soil scientist has cooperated with South Dakota State University on land that had been seeded to an alfalfa-brome mixture for 6 years before 1990. Then they began corn production using no-till, chisel, and moldboard plow management and measured the differences in water runoff and soil erosion. Under 2-1/2 inches of water applied during a 1-hour period, 2 days in a row, there was no runoff from no-till, while runoff from tilled treatments ranged from 20 to 50 percent. For 4 successive years in corn production, the no-till plots retained the high infiltration established by their alfalfa-brome planting, which was similar to that of some CRP lands.

National Soil Tilth Laboratory, Ames, Iowa.

10 SCS and ASCS helped the lab select paired plots of highly erodible soils that had similar management histories until half were enrolled in CRP. Scientists are documenting how CRP lands have changed during their retirement from cropping. They will also find which factors are most important in determining whether soil quality is improving or becoming degraded, so that the paired plot study results can be applied to other areas.

So far, under unusually wet conditions last fall, it looks like microbial biomass, nitrate, total nitrogen, total carbon, and wet soil aggregate stability may be reliable soil quality indicators.



National Sedimentation Laboratory, Oxford, Mississippi.

11 Scientists are evaluating management systems for erosion control in cotton production for small farms in hilly areas of the South. Acceptable stands and good yields were obtained in the first year, and 2 more years' testing are planned. Initial results indicate that cotton can be a profitable crop when grown with minimum tillage operations and winter cover—a necessity in this erosion-prone area.

Southern Piedmont Conservation Research Laboratory, Watkinsville, Georgia.

12 About 95 percent of CRP-enrolled land in the Southern Piedmont region of the United States is planted to trees, with the rest seeded to fescue grass. Given the current economic situation, most farmers will keep the grass and use it for livestock grazing. This will provide more income than annual cropping. And because of low grain prices, farmers will continue to keep land in trees, waiting for them to mature so they can be sold as pulpwood for making paper when they are 15 years old, or as saw timber when they are about 25.

For those keeping fescue grass, scientists at the Georgia lab have determined methods that promote long-term fescue production, as well as livestock weight gains, reproductive performance, and health.

Nutrient Deficiency Unleashes Jekyll-Hyde Virus

What provokes a normally mild-mannered virus to turn into a rampaging pathogen that leaves the heart muscle cells of mice broken and scattered?

The answer to that question—a severe nutritional deficiency—may well bridge a gap between two sciences that until now have enjoyed only a nodding acquaintance.

ARS nutrition chemist Orville A. Levander collaborated with University of North Carolina virologist Melinda A. Beck and others to show that a deficiency in either of two antioxidant nutrients—selenium or vitamin E—is enough to trigger a mutation in a normally benign human virus in test mice.

The researchers believe this is the first report of specific nutritional deficiencies permitting a nonvirulent virus to become virulent. It demonstrates the important role of nutrition in determining the severity of a viral infection.

And it could have widespread ramifications, they suspect. Other researchers have suggested that poor antioxidant status influences the course of influenza, hepatitis, or HIV infections.

Selenium is a critical component of the antioxidant enzyme glutathione peroxidase. The enzyme and vitamin E both protect fat-rich structures of cells—particularly cell membranes—against oxidative damage, explains Levander. An expert in both nutrients, he oversees research on nutrient requirements and functions at the Human Nutrition Research Center in Beltsville, Maryland.

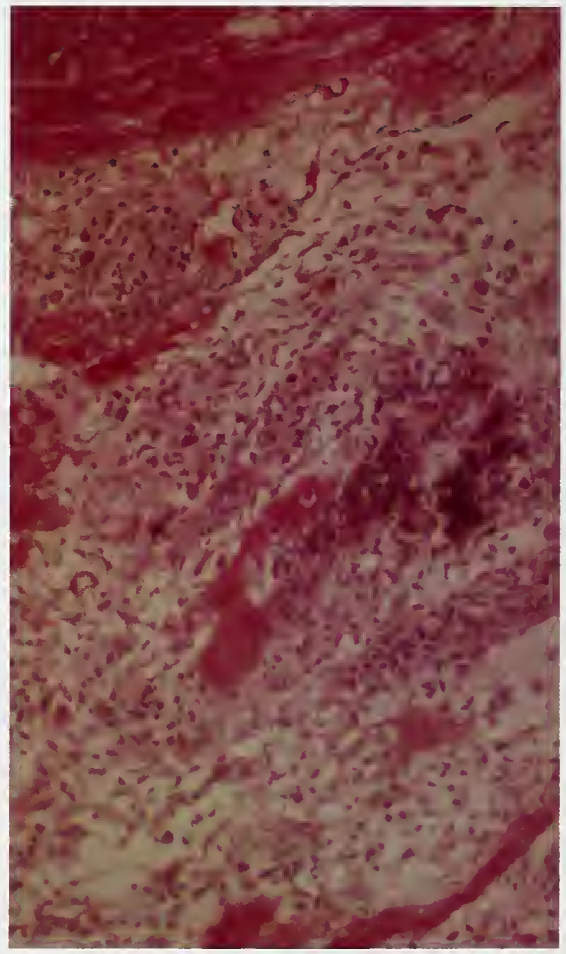
The antioxidants' job is to prevent oxygen free radicals and peroxides generated by normal metabolism from setting off chain oxidation reactions that could wreck the function and integrity of cells, Levander says. The two molecules perform the same service but work a little differently, thus serving as a backup to one another.

The background for this research is set in the Keshan region of China, where soils are nearly devoid of selenium.

Levander says thousands of infants, children, and women of child-bearing age living in the Keshan area suffered from heart damage until the government began widespread supplementation. He noted that supplementation has to be done carefully, as selenium can be quite toxic.

MELINDA BECK

MELINDA BECK



Since plants don't need the element to survive and grow, crops raised in Keshan area soils are also nearly devoid of selenium. So are the people who live in the area, because the Chinese eat food grown in their own locales.

Such geographically based nutrient deficiencies would be hard to find in the United States and other countries where the diet is homogenized by a nationwide food distribution system.

In this country, the Recommended Dietary Allowance for selenium after the first year of life is only between 20 and 70 micrograms (millionths of a gram) per day, depending on age and gender.

Clinically, the affliction known as Keshan disease "looks like congestive heart failure, except that it occurs in young children," says Levander.

Although selenium intake has been inextricably linked with Keshan disease and the addition of selenium to the diet has abated its occurrence, Chinese scientists had noticed some peculiarities that could not be explained by low selenium status alone, he says. For instance, its occurrence varied depending on the season and from one year to another. It looked like there might be an infectious component to Keshan disease.

Closeups show heart tissue removed from two mice infected with a normally benign strain of Coxsackievirus.

Far left: Tissue from the mouse receiving adequate dietary selenium appears normal. (K5534-1)

Near left: A selenium-deficient diet led to extensive myofiber damage, necrosis, and inflammatory infiltration. Similar heart tissue damage would have occurred if the mouse had consumed a diet deficient in vitamin E. (K5534-2)

The Chinese scientists isolated Coxsackieviruses and related enteroviruses in the blood of Keshan patients. Named after Coxsackie, New York, where they were first isolated, Coxsackieviruses are known to attack heart muscle, says Beck.

Levander says the Chinese scientists suspected the B4 strain of Coxsackievirus, because it aggravated heart muscle damage in test mice that were selenium deficient.

The U.S. researchers took the concept a giant step further, asking: Would a normally benign strain of the virus convert to a pathogen in a selenium-deficient host?

Beck and colleagues at Chapel Hill, North Carolina, obtained a benign strain of Coxsackievirus—B3—from researchers at the University of Nebraska Medical Center in Omaha. They inoculated mice that had proved to be a good model for studying this virus.

Surprisingly, the animals that had been raised on a selenium-deficient diet suffered heart muscle damage, just as if they had been given the virulent B4 strain of the virus. The group raised on a selenium-adequate diet, however, had normal hearts.

"We're changing the response of the host to the virus by changing the type of diet we're feeding," notes Levander.

But the results of the next phase of the experiment were even more surprising. When Beck inoculated with the virus she recovered from selenium-deficient animals into a new group that had gotten ample selenium in their diets, these animals also suffered heart muscle damage.

Apparently, the virus had mutated during its sojourn in the selenium-deficient animals. This change did not occur in animals that got ample selenium, she says. She is now sequencing the viral genes, looking for mutations.

Beck and company repeated these experiments in mice that were deficient in vitamin E, instead of selenium, and got the same results.

"I don't think Coxsackieviruses are unique," says Beck. "We may be getting new variants for other viruses due to extreme nutritional stresses."

She is now looking for nutritionally prompted mutations in more likely candidates—influenza viruses. "That's what influenza viruses do for a living—mutate," she says.

This new aspect of nutrient and gene interactions gives scientists a new perspective on viral infection, says Jacqueline Dupont, ARS national program leader for human nutrition research. For some 50 years after Pasteur's discoveries, it was thought that all disease was due to infection by microorganisms. Then, in the early 1900's, discovery of the cause of pellagra showed that diseases could also result from nutritional deficiencies.

"Now, we have a third phase showing an interaction between nutrition and infectious processes," says Dupont.

Beck says she doesn't know exactly how the deficiencies trigger a mutation, but she found that the deficient animals had much higher levels of the virus than the adequate animals. The higher replication rate alone increases the odds for a mutation to occur, she notes.

Also, immune function tests showed that some defense mechanisms were weakened in the deficient animals. That can give the virus "breathing room" to multiply.—By **Judy McBride,** ARS.

Orville A. Levander is at the USDA-ARS Beltsville Human Nutrition Research Center, Bldg. 307, 10300 Baltimore Ave., Beltsville, MD 20705-2350; phone (301) 504-8504, fax (301) 504-9062.

Melinda A. Beck is at the Frank Porter Graham Child Development Center, University of North Carolina, Chapel Hill, NC 27599; phone (919) 966-6809, fax (919) 966-7532. ◆

Exploring the Secret Life of Seeds

A refrigerator may not seem like a standard sleuthing tool, but it's a key part of ARS biologist James A. Young's detective kit when he spies on native plant seeds.

His goal: to learn how to make them germinate.

Young has already succeeded with hundreds of species, most native to the intermountain area between the Sierra-Cascade and Rocky Mountains in the Great Basin.

MICHAEL THOMPSON

Four-wing saltbush. (K5537-9)

He heads the ARS Conservation Biology of Rangelands Laboratory in Reno, Nevada.

Consultant John McLain, co-owner of Resource Concepts, Inc., in Carson City, Nevada, is a typical beneficiary of Young's scientific detective work. Using suggestions from Young, McLain's firm has helped the Santa Fe Pacific Mineral Company determine the best plants to reclaim the Lonetree Gold Mine near Winnemucca, Nevada. Lonetree is one of 120 active surface mines and quarries in Nevada that cover some 70,000 acres.

Federal and state environmental legislation requires that large projects, such as mines, mitigate their environmental impacts.

As each area within Lonetree is mined, tens of thousands of cubic yards of overburden—the material above a mineral deposit—are piled up. Each pile is later graded and replanted with plants native to the area, if possible.

"Mining has mixed a lot of different soil types, and in some cases we know very little about the plant communities that have been disturbed and how to reestablish them," McLain says. "Jim Young's efforts help us do it right the first time, which is a real cost savings to our clients."

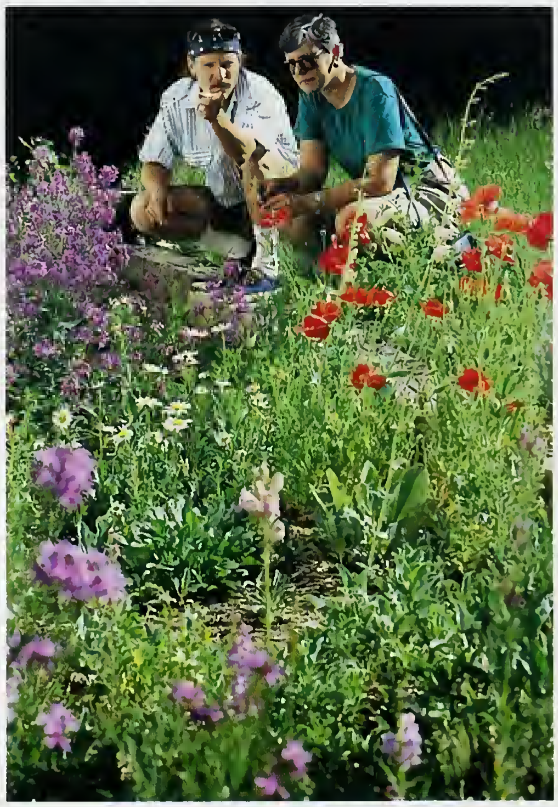
The challenge, says Young, is that native plant seeds have evolved into a myriad of different forms, while crops have been developed to be as uniform as possible. This diversity can make it hard to even find the seed.

MICHAEL THOMPSON

Boise bitterbrush. (K5537-7)

"The seed might be hairy or fuzzy, not smooth like a corn kernel," Young says. Four-wing saltbush seeds hide inside a winged, papery fruit. Indian ricegrass seeds come in five different colors and sizes. Other seeds have delicate structures that must be left intact for the seed to germinate. "A mistake at any stage of collecting or processing can jeopardize the ability of the seeds to germinate," Young says.

MICHAEL THOMPSON



Comstock Seed Company owner Ed Kleiner discusses native perennial wildflower plantings with ARS' Debra Palmquist. (K5535-1)

For those who master the skill of collecting and processing viable seeds, the rewards can be great. A pound of Indian ricegrass seed sells for up to $15; some other natives sell for up to $40 per pound. A comparable domestic seed like crested wheatgrass sells for $3 per pound.

"Many farmers have found native plant seed collection and processing to be an excellent way to supplement their income," Young says. Over 100 companies have also entered the business during the last decade.

Seeds don't do any good if they won't germinate. And each plant needs a unique combination of environmental conditions for its seeds to begin to grow. After thousands of hours of research, Young and his colleagues developed the Wildland Seed Laboratory algorithm, a systematic approach for testing plant seeds.

Once Young gets a seed to germinate, ARS mathematician Debra E. Palmquist and University of Nevada student Shannon Juarez take over. Juarez separates thousands of seeds into experimental groups; puts them in petri dishes, sand, or other substrate; and places them in 10 refrigerators. But like James Bond's ink pen—which doubles as a gun—Young's refrigerators are anything but ordinary.

The refrigerators are actually specialized germinators that provide 55 combinations of day and night temperatures ranging from 0°C to 40°C (32°F to 104°F).

Seeds are kept in darkness and exposed to simulated daytime

temperatures for 16 hours and to simulated nighttime temperatures for 8. The combinations are based on field measurements in the seeds' native habitats.

Once a week, for 4 weeks, Juarez records the number of seeds that germinate. Palmquist then takes the 4-week total for each temperature combination and develops a mathematical equation called a profile. The profile quantifies the relationship between temperature and seed germination for each plant.

According to Young, this approach is often sufficient to answer a question about a specific plant.

For example, "One of the most unusual requests this year is from several people who want to know how to grow a certain species of buckwheat, so they can raise a particular native butterfly," he says.

But commonly—after a wildfire, for example—a landowner asks more generally about which plants to grow to revegetate a large area. With Young's extensive knowledge, he can easily narrow the search down to a few plants. Then Palmquist statistically compares the narrowed-down field to identify the top performer.

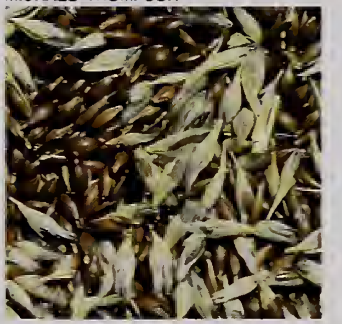
But standard statistical techniques only allow her to compare two profiles at a time. If she's considering a dozen plants, she'd have to make 66 comparisons. To simplify—and increase the accuracy—of this time-consuming process, she developed a new technique to compare all the profiles at once. Palmquist can then tell the landowner which plants should grow best in a specific location.

MICHAEL THOMPSON

Winterfat. (K5537-3)

Young and Palmquist have developed germination/temperature profiles for over 600 species of seeds. As an Associate Laboratory for the Association of Official Seed Analysts, Young's lab contributes to the standardization of testing procedures for the seeds. When a plant such as Indian ricegrass becomes commercially valuable, these standards provide buyers with consistent germination information on the seed tags.

MICHAEL THOMPSON



Indian ricegrass. (K5537-1)

Young's first foray into seed germination was designed to eliminate—not propagate—a plant. "In the 1960's, our major project was cheatgrass control," he says, "because you can't establish something until you get rid of the weeds."

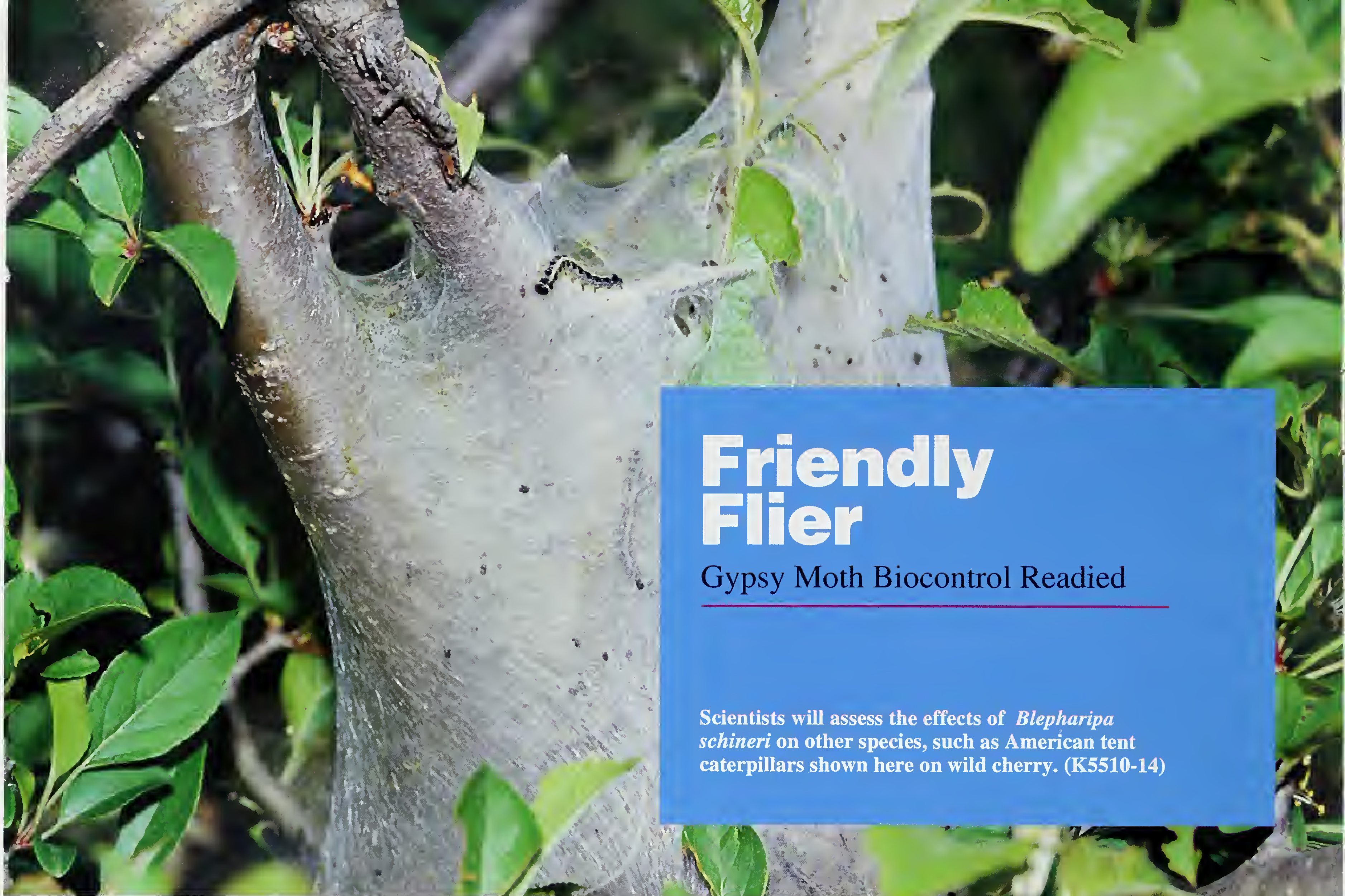
By studying how cheatgrass germinates, Young learned that the weed population in the seedbed could be reduced by removing the plant litter that accumulated during the year. "All current cheatgrass management programs are based on seed biology research conducted by ARS," Young says. He continues to study the germination requirements of rangeland weeds for ideas about how to control the weeds.

And like any good detective, Young doesn't give up. Though the keys to germinating a certain few seeds have eluded him for 30 years, he's still looking for clues to their secret lives.—By **Kathryn Barry Stelljes,** ARS.

MICHAEL THOMPSON

Mathematician Debra Palmquist notes the distribution of native range plants at a revegetation site near Hallelujah Junction, California. (K5536-1)

James A. Young and Debra E. Palmquist are at the USDA-ARS Conservation Biology of Rangelands Laboratory, 920 Valley Rd., Reno, NV 89512; phone (702) 784-6057, fax (702) 784-1712. ◆



Friendly Flier

Gypsy Moth Biocontrol Readied

Scientists will assess the effects of *Blepharipa schineri* on other species, such as American tent caterpillars shown here on wild cherry. (K5510-14)

SCOTT BAUER

A friendly fly imported from forests in Korea and Germany may soon help protect America's trees and shrubs from one of their worst insect enemies—gypsy moth.

ARS scientists expect the fuzzy, half-inch-long *Blepharipa schineri* fly to soon join the ranks of other beneficial insects brought into the United States to combat the destructive gypsy moth. Black with brown eyes and faintly reddish bands on its sides, the fly is harmless to humans.

Gypsy moths infest parks, woodlands, and backyards in 16 northeastern states. In spring and summer, they feed on foliage of hundreds of different kinds of trees and other greenery. Many defoliated trees weaken and die.

A parasite of the gypsy moth, the *B. schineri* fly kills unlucky gypsy moth caterpillars that accidentally eat fly eggs while munching on leaves. A gray-black speck about the size of a thumbtack point, the fly egg hatches inside the caterpillar's stomach. The transparent *B. schineri* maggot that emerges will later poke holes in the caterpillar's gut, then wriggle its way to the nerve cord that runs the length of the body.

While the gypsy moth caterpillar transforms into a pupa—a pre-moth that slumbers in a silky, loosely woven cradle—the *B. schineri* maggot feeds on the host's innards, slowly killing it.

In early summer, the fully grown maggot cuts a slit in the now empty pupal shell, then falls to the ground. Within a final layer of hardening skin, the maggot also forms a pupa. The skin becomes a home, or puparium, until the next spring, when the insect makes its amusing debut as a fly.

"A fly that has just climbed out of its puparium looks as if it has a tiny balloon on its head. Actually, it's an inflatable chamber. The fly fills it

SCOTT BAUER

A female *Blepharipa schineri* fly lays her eggs on foliage. (K5507-3)

SCOTT BAUER

Gypsy moth caterpillars are the No. 1 forest and shade tree pest in the Northeast. (K5504-7)

SCOTT BAUER

Entomologist Roger Fuester inspects rearing cartons for gypsy moth parasites. Newly imported natural enemies such as *Blepharipa schineri* are kept in quarantine until they are proven safe to release. (K5505-3)

Gypsy moths infest parks, woodlands, and backyards... feeding on foliage of hundreds of different kinds of trees and other greenery.

with blood, forcing the puparium to crack open so the fly can escape."

After mating, the female *B. schineri* fly lays her eggs on leaves that gypsy moth caterpillars have begun to browse.

Female gypsy moth caterpillars are 1-1/2 to 15 times more likely to eat the eggs—and be killed by the parasite—than males. It's a matter of exposure: Females remain caterpillars for about a week longer than their male counterparts.

Males skip this extra step, or instar, and may already be sheltered in their pupal cradles while females are still feeding.

Hungry *B. schineri* maggots can't stop ravenous caterpillars from defoliating trees and shrubs. But the flies can lessen the following year's gypsy moth generation by killing pupae. Parasitized pupae will never become fertile, egg-laying moths.

Tomorrow's first supplies of the *B. schineri* flies for American forests may come from an insect nursery, or insectary, at the ARS Beneficial Insects Introduction Research Unit in Newark, Delaware.

Entomologist Roger W. Fuester and coworkers nurture the *B. schineri* that exit from some 1,000 parasitized gypsy moth caterpillars.

The caterpillars were painstakingly harvested from forests near Seoul, Korea, by Robert W. Pemberton and colleague Jang-Hoon Lee. Both were with the ARS Asian Parasite Laboratory in Korea.

They picked the caterpillars from more than a half-dozen different kinds of trees, including birch, larch, poplar, cherry, and oak.

In Korea, says Pemberton, gypsy moth populations seldom burgeon, thanks to *B. schineri,* a phalanx of other gypsy moth parasites, and a natural virus that slays the moth.

B. schineri is the third most successful insect parasite of Korea's gypsy moths, according to an exacting, 5-year study by Pemberton, Lee, and co-investigators David K. Reed, Robert W. Carlson, and Ho-Yeon Han.

The two top-ranked parasites in their study—the *Cotesia melanoscelus* wasp and the *Parasetigena silvestris* fly—already live in the United States. They were imported in the early 1900's to battle the gypsy moth.

The rankings result from the researchers' post-mortem survey of more than 50,000 parasitized gypsy moth caterpillars and pupae. The three leading parasites were the most numerous of those that came out of caterpillars brought indoors and raised by the researchers and their co-workers at the Seoul laboratory. The study—one of the most extensive investigations ever conducted of gypsy moth's natural enemies—confirmed the importance of the *Cotesia* wasp and *Parasetigena* fly.

SCOTT BAUER

Large feet easily distinguish male *Blepharipa schineri* flies from females. (K5509-6)

Besides husbanding the Korean flies furnished by Pemberton and Lee, Fuester and colleagues safeguard *B. schineri* that live inside gypsy moth pupae collected in Europe. Research entomologist Franck Hérard found the pupae on oak trees in Germany. He works at the ARS European Biological Control Laboratory in Montpellier, France.

Raising Flies for 60 Million Acres

At Newark, researchers refine the complex process of raising new generations of healthy flies. When the insect gains federal and state approvals, says Fuester, lab-reared *B. schineri* may be set free in the woods to help keep gypsy moth in check. That might happen as early as 1995.

That would be good news for homeowners and others in the East, where the moth is established over some 60 million acres. What's more, the fly might aid other states where the moth has spread. And it may also prove a welcome warrior in states newly threatened by the European gypsy moth's "evil twin," the Asian gypsy moth.

B. schineri's homelands cover an enormous expanse—Europe through Asia—where it is an effective natural enemy of both the European and Asian gypsy moths.

That's particularly important to Pacific Northwest states, where the Asian gypsy moth has been an occasional and unwelcome visitor. Hitchhiking on ships, the moth has jumped ashore at port cities such as Tacoma, Washington, and Portland, Oregon, prompting emergency efforts by ag officials to thwart infestations.

What might happen if *B. schineri* is set free in the United States?

"Chances are you wouldn't even notice the new fly," predicts Fuester. "*B. schineri* flies are unobtrusive. They live quiet lives, sipping nectar, mating, and laying eggs.

"If you're out fishing or hiking in the woods, they may land gently on you. But they won't sting or bite. They buzz, but it's not a loud buzz, so it isn't annoying.

The fly's best use, says Fuester, may be to quell pockets of gypsy moth infestations, before they become outbreaks. And the kills scored by *B. schineri* should complement the efforts of other biocontrols, including a relative—the *B. pratensis* fly—and a virus.

A U.S. resident since 1907, *B. pratensis* is one of more than 50 insects brought here during the past eight decades by entomologists seeking to squelch gypsy moth by natural means. Recently, however, researchers from New Jersey, Pennsylvania, and New York have noted a worrisome trend: *B. pratensis* is losing its edge. Fuester hopes that *B. schineri* will compensate for its cousin's decline.

No one expects *B. schineri*—or any single gypsy moth parasite—to suddenly become the overnight hero that clobbers the moth. But *B. schineri* may prove a valuable new player on the American biocontrol team.—By **Marcia Wood,** ARS.

Robert W. Pemberton is now with the USDA-ARS Aquatic Weed Control Research Unit, 3205 College Ave., Fort Lauderdale, FL 33314; phone (305) 475-0541, fax (305) 476-9169.

Jang-Hoon Lee is at the USDA-ARS Plant Science and Water Conservation Laboratory, 1301 N. Western St., Stillwater, OK 74075; phone (405) 624-4407, fax (405) 372-1398.

Roger W. Fuester is in the USDA-ARS Beneficial Insects Introduction Research Unit, 501 South Chapel Street, Newark, DE 19713; phone (302) 731-7330, fax (302) 737-6780.

Franck Hérard is stationed at the USDA-ARS European Biological Control Laboratory, BP 4168 Agropolis, 34092 Montpellier, Cedex 5, France; phone 33-67-04-56-00, fax 33-67-04-56-20. ◆

ROGER FUESTER

The tiny eggs of *Blepharipa schineri* (two at leaf edge) must be eaten by gypsy moth caterpillars for parasitism to occur. (K5511-1)

Extrafloral Nectaries May Help Plant Defense

With tiny caches of sugary nectar, plants offer a food bribe to helpful insects. In return for this sweet, high-energy food, the friendly insects may help protect plants from harmful ones.

Scientists term the inconspicuous storehouses "extrafloral nectaries" because they're located away from flowers—the usual source of nectar. Typically, the nectaries are found on the tops of leaves where the leaf blade and stem meet, says ARS entomologist Robert W. Pemberton.

Often smaller than a grain of salt, extrafloral nectaries are glands that may be shaped like miniature cups or pegs.

They've been known to science for more than a century, perhaps most notably for their mutually beneficial use by ants that guard plants from attack by other insects.

Now, a recent study by Pemberton and colleague Jang-Hoon Lee suggests that a similarly beneficial relation, or mutualism, may occur among plants with extrafloral nectaries and another important group of insects—helpful parasites.

The findings may open the door to new strategies for managing desirable parasites and boosting their effectiveness in protecting plants.

Pemberton and Lee scrutinized extrafloral nectaries in a 2-year study of gypsy moth parasites in forests near the ARS Asian Parasite Laboratory in Seoul, Korea.

ROBERT PEMBERTON

Extrafloral nectaries such as these on a peach leaf *(Prunus persica)* secrete nectar that helps attract beneficial insects. (94-81)

The researchers collected more than 3,500 gypsy moth caterpillars and pupae for the experiment. They plucked them from poplar, viburnum, black locust, wild cherry, and other plants equipped with extrafloral nectaries, as well as from species growing nearby that lack these special structures—oak, chestnut, maple, and rhododendron.

The two most important species of gypsy moth parasites in Korea, the *Cotesia melanoscelus* wasp and the *Parasetigena silvestris* fly, killed twice as many caterpillars and pupae on plants with these sugar glands, report the scientists.

Parasitism by a third key parasite, the *Blepharipa schineri* fly, was slightly higher on the plants with extrafloral nectaries, but not significantly so.

"These nectaries may put the beneficial parasites in the vicinity of the leaf-eating caterpillars more frequently than occurs on plants without the nectaries," says Pemberton. "That could increase the caterpillar's odds of getting zapped."

When a parasite strikes, caterpillar death is certain, though parasite modes of attack vary. The stingless *C. melanoscelus* wasp, for example, attacks the caterpillar by inserting an egg into it. The egg hatches into a wasp larva that feeds on caterpillar innards. Later, the wasp emerges from the dying or dead caterpillar and develops into a winged adult that begins the cycle again.

The wasp and fly belong to the team of parasites recruited to fight gypsy moths in American forests, city parks, and backyards.

More needs to be known about the relationship that has evolved between these parasites and the plants that bear extrafloral nectaries, the researchers say. In the meantime, Pemberton and Lee recommend releasing the recruits in environments that have an abundance of plants with extrafloral nectaries.—By **Marcia Wood,** ARS.

MLO's—Invisible Crop Destroyers

Using new diagnostic techniques, ARS scientists are tracking down invisible, highly destructive crop pests called MLO's—mycoplasmalike organisms.

Researchers at the ARS Molecular Plant Pathology Laboratory in Beltsville, Maryland, developed the techniques, which could lead to fast, accurate tools to detect MLO's—and the insects that carry them.

"We first began developing the molecular techniques several years ago," says plant pathologist Robert E. Davis, who heads the laboratory. "They offer a whole range of uses, the most important of which is in creating a much-needed scientific base of information on MLO's."

First discovered in 1967 by Japanese scientists, MLO's have since been linked to diseases that devastate orchard-grown fruits like apple, peach, and pear. They are also the culprits responsible for diseases of vegetables, ornamental nursery crops, and landscape trees including the dogwood and American elm. Afflicted plants show a variety of symptoms, such as leaf curl, yellowing, wilting, and death.

MLO's are thought to cause problems all over the world. They are known to be spread by feeding insects and through plant propagation.

Until recently, detecting MLO's in crops or imported plant stock usually meant several weeks', months', or even years' worth of greenhouse testing. In such tests, material from potentially infected plants is grafted onto "guinea pig" plants to reveal disease symptoms.

Now, detection could only be a matter of days, thanks to genetic technologies being developed at the Beltsville lab.

"We're hoping these can be used on plant germplasm to speed up the clearance process at plant quarantine operations," Davis says. "We also hope they will speed the detection of insects that spread MLO diseases."

That could prove a boon to growers needing immediate information on where or when to spray for MLO-carrying insects, like leafhoppers, in crop fields.

REGIS LEFEBURE

Plant pathologists Ing-Ming Lee (left) and Robert Davis compare tissue-cultured paulownia shoots. Mycoplasmalike organisms have stunted the one on the left. (K5539-1)

To detect an MLO or characterize its disease, the researchers use an array of techniques, including polymerase chain reaction and restriction fragment length polymorphism.

With these techniques, ARS plant pathologist Ing-Ming Lee, Davis, and colleagues have studied genetic similarities among the organisms and devised a classification scheme for different strains. Lee says the scheme provides them with a guide for uncovering genetic evidence that a particular MLO is present within a plant or insect specimen.

Applied to DNA samples from either, the technologies enable researchers to detect and identify gene sequences that characterize the MLO, if it is present.

"An advantage of these methods," says Lee, "is the sensitivity and speed with which we can both detect an MLO and identify it, using the lab's classification system."

Adds Davis, "We hope commercial companies interested in these approaches will eventually bring the technology to a level that will be used by plant disease diagnosticians and Extension Service people working with growers."

That's also the idea behind a computer model now being built by scientists at the Ohio Agricultural Research and Development Center in Wooster, about 90 miles northeast of Columbus.

There, researchers Casey Hoy, Sally Miller, and Lowell "Skip" Nault are using the ARS techniques to compile a model with lab and field information about MLO strains that cause aster yellows—a disease of carrots, lettuce, and celery grown in Ohio and other midwestern states.

Hoy says the model is designed to simulate aster yellows outbreaks in the crops, as well as show how controls targeting both leafhopper carriers and the MLO affect progress of the disease.

Miller is using the ARS genetic technology to verify that leafhopper and plant specimens sampled for their research actually have the MLO. She's also identifying which strains pose a threat to Ohio's vegetable crops.—By **Jan Suszkiw**, ARS.

Robert E. Davis and Ing-Ming Lee are at the USDA-ARS Molecular Plant Pathology Laboratory, Rm. 252, Bldg. 011A, 10300 Baltimore Ave., Beltsville, MD 20705-2350; phone (301) 504-5745, fax (301) 504-5449. ◆

Science Update

More on Envira's Fire Ants

An engineer who was part of the scientific team that traveled to Envira, Brazil, last year [see "Fighting the Fire Ant," *Agricultural Research,* January 1994, pp. 4-9] says preliminary reports indicate that efforts to fight the fire ants have met with initial success. Brazilian officials estimate that fire ant populations declined by 80 percent after the second application of a bait containing an insect growth regulator. USDA scientists helped apply 500 pounds of Logic donated by Ciba-Geigy in September 1993, and Brazilian officials applied another 500 pounds in February/March 1994.

The trip by scientists from ARS and the Animal and Plant Health Inspection Service was made possible through funding and travel arrangements by USDA's Foreign Agricultural Service. The government of Brazil and the state of Amazonas paid for travel and other expenses within Brazil.

The USDA research team hopes to return to Envira in the fall of 1994 to conduct a more thorough evaluation of the fire ant control effort there and to promote a collaborative research project with Brazilian scientists for future methods of controlling fire ants in the Amazon. *Danel Haile, USDA-ARS Medical and Veterinary Entomology Research Laboratory, Gainesville, Florida; phone (904) 374-5928.*

First Artificial Wasp Diet Released

In May, ARS scientists began the first field test of an insect parasite produced on a lab diet containing no component of its insect host. The parasite, the *Catolaccus grandis* wasp, attacks boll weevil larvae. Female wasps lay eggs near the larvae, and the offspring hatch and feed on the pests. In ARS lab and field tests since 1992, the wasps have cut weevil numbers up to 90 percent. But those tests used wasps lab-raised on live weevils. For the new test, scientists released wasps fed only on the artificial diet, a yellow jellylike mix of amino and fatty acids, sugar, vitamins, and a protein source. The diet is the most crucial part of technology needed to mass-produce the wasp and other parasites as alternatives to chemical insecticides. ARS is seeking a patent on the diet and has begun cooperating with a U.S. Department of Energy facility to develop a prototype mass-production system for the wasp. *Edgar G. King, USDA-ARS Subtropical Agricultural Research Laboratory, Weslaco, Texas; phone (210) 565-2423.*

Crossbred Cattle Less Affected by Fescue Fungus

Genetic diversity helps cows and calves cope better with natural toxins in tall fescue infected by endophyte fungus. This pasture grass grows on 35 million U.S. acres. But cattle that graze on fungus-infected tall fescue can suffer poor growth and reproduction and painful circulatory problems. In a 3-year study, cows—purebred Angus or Brahman or a mix of the two—were bred to Polled Hereford bulls and grazed on fungus-infected tall fescue. At weaning, three-breed calves (Polled Hereford-Angus-Brahman) weighed 16 percent more than two-breed calves. And, crossbred cows weaned 26 percent more calves than purebreds. The study was done in cooperation with the University of Arkansas. *Michael A. Brown, USDA-ARS South Central Family Farms Research, Booneville, Arkansas; phone (501) 675-3834.*

Inactive Microorganism Breaks Down Milk Sugar

A bacterium long used to make cheese and yogurt has now been harnessed as a convenient way to lower the milk sugar, or lactose, in dairy products. Two-thirds of American adults can't digest the lactose, but ARS scientists have patented a bacterium that can. It is an inactivated form of a food-grade bacterium, *Streptococcus thermophilus,* but the lactose-eating enzyme it produces remains active nonetheless. The enzyme may be added to milk before pasteurization. This new approach thus provides an edge over similar, costlier enzymes extracted from yeasts and fungi. *George A. Somkuti, USDA-ARS Eastern Regional Research Center, Philadelphia, Pennsylvania; phone (215) 233-6474.*

ARS and Biotech Firm Tackle Postharvest Rot in Potatoes, Tomatoes

ARS and a private firm seek to genetically engineer potatoes and tomatoes for more resistance to postharvest diseases, under a cooperative R&D agreement. The diseases—including bacterial soft rot, ring rot, and blackleg—cost more than $400 million a year in commodity losses. Demeter Biotechnologies, Ltd., in Research Triangle Park, North Carolina, is supplying its lab-designed genes for antibacterial proteins. ARS scientists are providing a gene fragment called a promoter to serve as an on/off switch for the genes. ARS and Demeter will also study ways to use molecular genetics to improve the nutritional value of potatoes. *William Belknap, USDA-ARS Western Regional Research Center, Albany, California; phone (510) 559-6072.*

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Upcoming in the SEPTEMBER Issue

- U.S. growers and consumers can look forward to having a bouquet of striking new floral crops to pick from.
- A NAFTA benefit: cooperative research with Mexico aimed at conserving erosion-prone lands on both sides of the border.
- Diet-conscious pizza lovers may soon enjoy a low-fat mozzarella with flavor, texture, and meltability approaching the "real thing."